



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

MEMORANDUM

JUL 12 2016

SUBJECT: Revised Review of the Valent Biosciences Corp., XenTari® *Bacillus thuringiensis* subsp. *aizawai* request ABTS-1857 for label amendment, Decision # 512930

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Thru: Shannon Borges, Acting Senior Scientist
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ACTION REQUESTED: Revised Review of the Valent Biosciences Corp., XenTari® *Bacillus thuringiensis* subsp. *aizawai* ABTS-1857 request for label Amendment Justification for the elimination of Aerial Application restriction on Rice and Cereal Crops, Decision # 512930, Submission # 979441, DP # 431763, MRID 49806201

CONCLUSION: Adverse effects to aquatic organisms are not expected as a result of the addition of teff to the current label. However, aerial spraying of XenTari® *Bacillus thuringiensis* subsp. *aizawai* ABTS-1857 is not recommended for flooded Teff (and other cereal grains cropped before Rice) or flooded rice beds due to the potential persistence of *Bt aizawai* spores and toxins in soil and aquatic ecosystems, and the potential negative impact on aquatic invertebrates and fish. Originally, estimated environmental concentrations (EEC's) were calculated without taking into account repeated applications, as are recommended on the current label, so exposure for nontargets may be greater than was previously accounted for in the 1997 Freshwater Aquatic Organism Risk Assessment for XenTari *Bt aizawai*. It may be possible to change the label to allow direct applications to flooded rice fields with a duration longer than 7 days required prior to release of flood waters. The registrant should specifically justify why 7 days is enough time to hold floodwaters. EPA recommends that Valent Biosciences discuss the required information with EPA prior to submitting additional data or rationale.

DATA REVIEW RECORD

Active Ingredient: *Bacillus thuringiensis* subsp. *aizawai* ABTS-1857

Product Name: XenTari®

Company Name: Valent Biosciences Corp.

DP Barcode: 431763

Decision No. : 512930

Submission No. : 979441

BACKGROUND:

Bacillus thuringiensis subsp. *aizawai* produces Cry1A, Cry1Ab, Cry1Ca, Cry1Da, Cry2ab proteins which are known to be particularly effective against army worms. During sporulation the bacterium produces a crystalline inclusion that has insecticidal protoxins. The crystalline inclusion ingested by insects is soluble in the alkaline midgut of insects which causes the release of the protoxin causing lysis of the gut epithelial cells and eventually death of the insect itself (Attathom et al. 1995).

EPA completed the Freshwater Aquatic Organism Risk Assessment for XenTari *Bt aizawai* on November 19, 1997¹. At that time the EEC with direct application to water was calculated as 4.70 mg/L. However, since the application to water directly was prohibited in that memo, an EEC was recalculated as 0.538 mg/L using the GENEEC environmental fate model, while assuming only one application and no accumulation in the environment. The effects of XenTari on *D. magna* and rainbow trout were summarized in the 1997 memo. The toxicity to *D. magna* was concluded to be due to a heat labile exotoxin produced during fermentation. Xentari then changed their manufacturing process and retested the active ingredient on Rainbow trout and *D. magna*. The Rainbow trout study was found to be Supplemental; Upgradeable, the *D. magna* study was Acceptable, and considered slightly toxic. Based on calculated risk quotients aquatic use needed to be prohibited to prevent hazards to aquatic organisms.

Recently published research has shown that Cry1Ab which is present in XenTari is toxic to *D. magna* and negatively affects development and reproduction (Bøhn, Rover et al. 2016), which further confirms the results from studies cited in the 1997 memo.

XenTari® is currently used on a variety of crops including vegetable, grains, tubers, legumes, and fruits for control various insect pests including armyworms, lepidopterans, loopers, leaf rollers, hornworms, cutworms, and diamondback moth (Refer to XenTari® label for complete list).

¹ Memorandum from M. Mendelsohn through Z. Vaituzis to P. Hutton. Subject: Freshwater aquatic organism risk assessment and revision of predatory wasp environmental hazards statement for XenTari (*Bacillus thuringiensis* subspecies *aizawai*), dated November 1997.

The addition of Teff has been added because Teff is becoming a more popular small grain, and growers need the option of using XenTari® to control lepidopterans. With respect to aerial applications into flooded rice, the registrant has stated that it wishes to offer rice farmers a biological option in order to replace synthetic insecticides.

Valent Biosciences Corp., has submitted a justification for the elimination of the aerial application restriction of XenTari® containing *Bacillus thuringiensis* subsp. *aizawai* ABTS-1857 on flooded rice beds and is seeking addition of Teff cereal crop to the label. The aerial application requested label amendment to flooded rice specifically states “For flooded rice – Do not release floodwaters within 7 days of application.”

EPA previously completed a review of XenTari® containing *Bacillus thuringiensis* subsp. *aizawai* ABTS-1857 on June 13, 2016²; this memo is a revised version of that memo which has deleted an incorrect statement regarding the percentage of a.i. present in the formulation. Additionally, this memo discusses aerial spraying to non flooded grain crops, and requests that the registrant justify that seven days is an adequate period of time for the degradation of flood waters containing *Bacillus thuringiensis* subsp. *aizawai* ABTS-1857.

Discussion

The registrant has presented an argument that aerial applications are no longer considered toxic to *Daphnia* because of information presented in the EPA *Bt* RED from 1998 (pg. 29-30) which states that the risk to aquatic invertebrates is considered minimal to nonexistent based on currently registered label use rates because the environmental concentration is lower than the observed laboratory effect levels. Additionally, the registrant has referenced (pg. 25) of the same *Bt* RED from 1998 where no toxicity or pathogenicity of *Bacillus thuringiensis* subsp. *aizawai* was seen in freshwater fish or estuarine/marine species. However, as noted in the 1997 risk assessment memo, the aquatic assessment for fish was not complete at that time, and the prohibition on application to water was present at the time the RED was published. The registrant claims that as a precautionary measure maintaining water exposed to *Bt* sprays for a 7 day minimum in the flooded rice field is sufficient to mitigate any possible risk to *Daphnia* or other aquatic organisms, and that application to rice floodwater will not result in an increase of *Bt* above natural background levels. The argument the registrant presents hinges on the 1998 RED document on *Bt* and on research manuscripts which discuss short half-life persistence of *Bt* in soil and aquatic ecosystems, no effects on aquatic invertebrates, and no increase in *Bt* titers above natural levels after spraying.

Using XenTari’s current maximum application rate of 2 lbs/acre at 54% active TGAI (1.08 lb TGAI/acre) the EEC per application is 0.091 mg/L (See Calculation of EEC #1 below), which is almost 6 times less than the original recalculated value of 0.538 mg/L taking into account GENEEC assumptions including one application only and no accumulation of Cry toxins and *Bt* spores. However, the proposed amendment would allow direct application to water, which

² Memorandum from Milutin S. Djurickovic thru Shannon Borges to Susanne Cerrelli, Subject: Review of the Valent Biosciences Corp., XenTari® containing *Bacillus thuringiensis* subsp. *aizawai* ABTS-1857 for label amendment, Decision #512930.

would result in an EEC of 1.21 mg/L (See Calculation of EEC #2 below). While the *Daphnia* acute 48-hour LC₅₀ of 34 mg/L would not result in an EEC exceeding the level of concern (0.05) for sensitive freshwater invertebrates (RQ = 0.04), a 10-day *Daphnia* study described in the 1997 memo indicated higher toxicity (LC₅₀ = 12 mg/L) results in an RQ of 0.10, which does exceed this level of concern. This value does not take into account repeat applications. The current label states that application of XenTari can be repeated if necessary at intervals between 3 to 14 days, therefore accumulation of *Bt* spores and Cry toxins in the flooded rice beds and surrounding ecosystems can be reasonably expected, and EECs may be higher than calculated herein.

Calculation of EEC #1³

$$\begin{aligned} \text{EEC (1.08 lbs TGAI/acre)} & (1 \text{ acre}/0.4047 \text{ ha})(454 \text{ g/lb})(10 \text{ ha}) = 12115.64 \text{ g} \\ 12115.64 \text{ g} (.10) & = \mathbf{1211.56 \text{ g}}. \quad 12115.64 \text{ g} (.05) = \mathbf{605.78 \text{ g}} \\ \mathbf{1211.56 \text{ g} + 605.78 \text{ g} = 1817.34 \text{ g}} \\ (1817.34 \text{ g}/1 \text{ ha})(1/2 \text{ m})(1 \text{ ha}/10,000 \text{ m}^2)(1000 \text{ cm}^3/1 \text{ L})(1 \text{ m}^3/1,000,000 \text{ cm}^3)(1000 \text{ mg}/1 \text{ g}) \\ & = \mathbf{0.091 \text{ mg/L}} \end{aligned}$$

Calculation of EEC #2⁴

$$\begin{aligned} (1.08 \text{ lbs TGAI/acre})(454,000 \text{ mg}/1 \text{ lb})(1 \text{ acre}/40,468,564 \text{ cm}^2)(1/10 \text{ cm})(1000 \text{ mL}/1 \text{ L}) \\ = \mathbf{1.21 \text{ mg/L}} \end{aligned}$$

Management of rice fields with respect to flooding can vary between the major rice producing states and according to meteorological conditions. Management practices for conventional rice production can have several flooding periods that can last up until harvest which encompasses a period of several months (2012 Texas Rice Production Guidelines). In organic rice production flooding may only last a short time and control of insect pests is done through flooding, and spot spraying for army worms before flooding based on scouting reports (Organic Rice Production NCAT). Additionally, farmers may rotate a wheat or other cereal such as a Wheat/Rice or Teff/Rice relay crop where the grain is flooded in milk stage, and after harvest, the grain stalks are used as a straw bed for the coming rice crop (Organic Rice Production NCAT). Therefore, spraying before rice is planted could occur in flooded Teff fields to prepare for rice. The label should clarify that application to flooded teff fields is not recommended.

EPA generally agrees that Cry toxins produced in transgenic crop plants are not persistent; however, some literature indicates that certain Cry toxins and Cry toxin degradants may persist in soil and water ecosystems for longer (Valdor et al. 2015). The registrant has cited manuscripts that report half-lives of the Cry1Ab toxin between 1.6 to 4 days, and has stated that application of *Bt aizawai* will not increase the natural titer of *Bt* species following application. However, whether the degradation of Cry toxins produced by genetically engineered crop plants is comparable to those produced by bacterial *Bt aizawai* is unclear, and the rationale does not address this point. Half-lives are insufficient to explain persistence of microbial *Bt* over time. The Bai study referenced in the rationale provided by the registrant was a lab study not a field

³ The GENEEC model assumed 10% and 5% would reach adjacent surface water due to runoff and drift, respectively. The GENEEC model calculated the amount of a pesticide that would reach a 1 ha body of water 2 m deep via runoff and drift from a surrounding treated area of 10 ha

⁴ Based on an assumption of direct application to a 10 cm body of water, consistent with assumptions of EPA's Tier I Rice Model

study and full degradation took 14 to 16 days. Further evidence has shown that Cry1A which is present in the XenTari formulation, can degrade in the field by 50%, but 20% of the toxin is still present at 28 days (Hung, Truong et al. 2016). This conflicting information is not discussed in the rationale submitted to support this amendment. Additionally, while most of the rationale rests on degradation of Cry proteins, the 1997 risk assessment noted that other toxins are present, which were determined to be the cause of the toxicity observed in aquatic nontarget testing. The environmental fate of these toxins is not known, and may differ from that of Cry proteins.

Given the potential persistence of the toxins present in *Bt aizawai* and the *Bt aizawai* itself, in addition to the potential for multiple applications, the rationale presented is insufficient to change EPA's position regarding risks to aquatic organisms and does not support the proposed amendment. Exposure sufficient to cause adverse effects could occur, especially with repeated spraying applications of XenTari, and that this could cause unreasonable adverse effects on aquatic invertebrates and fish.

The submitted rationale does not support the amendment as requested. While there is literature that discusses degradation of Cry proteins within shorter cycles, there is also literature describing longer degradation time cycles. Also, limited information was provided to support the degradation of *Bt aizawai* and *Bt aizawai* spores. It may be possible to change the label to allow direct applications to flooded rice fields with a duration longer than 7 days required prior to release of flood waters with sufficient justification addressing environmental fate of *Bt aizawai* and its spores and toxins. However, the registrant must specifically justify why 7 days (or some other duration) is enough time to hold floodwaters to ensure that adverse effects to aquatic organisms will not occur, taking into consideration multiple applications and uncertainties described above. Any data gaps identified in previous risk assessments must also be addressed. EPA recommends that Valent Biosciences discuss the required information with EPA prior to submitting additional data or rationale.

References

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